Molecular identification of *invA* gene from *Salmonella* Species Isolated from human sources in SouthernTaraba State, North-East Nigeria

ABSTRACT

Place and Duration of the Study: The study was conducted in Wukari, Donga, Ibi, and Takum Local Government Areas in Southern Taraba State, North-East Nigeria. These areas were chosen due to their high population of farmers and traders, making them suitable locations for investigating foodborne pathogens. The experimental research spanned a period of 7 months, during which 200 blood and stool samples were collected from food vendors in the selected areas.

Methodology: Sample collection involved the collection of 200 blood and stool samples from food vendors in Wukari, Donga, Ibi, and Takum Local Government Areas. Isolation and confirmation of *Salmonella* species were carried out through cultural and biochemical analyses, with reference strains used for quality control. Deoxyribonucleic Acid (DNA) extraction was performed using the boiling technique, followed by Polymerase Chain Reaction (PCR) amplification of the invA gene. Electrophoresis on an agarose gel was used to visualize the presence of the invA gene in the isolates.

Results: The results of the study revealed a high prevalence of Salmonella species among food vendors in Southern Taraba State, North-East Nigeria. Analysis of the *invA* gene showed its presence in all isolates, indicating the widespread distribution of virulent strains in the study area. The findings underscore the importance of PCR-based methods for the detection of Salmonella and suggest the need for comprehensive surveillance and control measures to prevent foodborne illnesses.

Conclusion: In conclusion, the study demonstrates the significant prevalence of *Salmonella* species among food vendors in Southern Taraba State, North-East Nigeria. The detection of the *invA* gene in all isolates highlights the potential virulence of these strains and emphasizes the importance of effective surveillance and control strategies to mitigate the risk of foodborne diseases. Further research is warranted to elucidate the genetic diversity and antimicrobial resistance genes associated with*Salmonella* strains in the region, facilitating the development of targeted interventions for public health protection.

Keywords: Salmonella, food vendors, prevalence, invA gene, PCR.

Introduction

*Salmonella*species that are responsible for typhoid fever and non-typhoidal gastroenteritis in humans are classified as Typhoidal Serovars (TS) and Non Typhoidal Serovars (NTS)[1]. The word “typhoid fever” coined from a Greek word typhus meaning “smoky” was first used by a French pathologist, Pierre-Charles-Alexander in the year 1829 to describe a delirium situation shown by patients as a reaction to a certain infection[2]. The infection was characterized by lesions in the abdominal lymph nodes of patients who had died from gastric fever [2]. In the year 1880, German pathologist Karl Joseph Eberth identified the causative organism of typhoid from spleen and abdominal lymph nodes of patients [3]. This was further confirmed in 1884 by a German bacteriologist, Georg Theodore August Gaffky who cultured and identified the organism as a confirmation to the earlier discovery [2]. *Salmonellatyphi* and paratyphi A, B, C is the etiological agents of typhoid fever, and paratyphoid fever respectively in humans while several species of the NTS *Salmonella* especially are responsible for gastroenteritis with *Salmonella Enteritidis* and *Salmonella Typhimurium*being the most responsible for *Salmonella* induced gastroenteritis [1]. However, cases of foul typhoid have been documented but it is caused by

SalmonellaGallinarum[4]. *Salmonella*is a rod-shaped, gram-negative, motile bacterium whose only reservoir is the human body [5]. *Salmonella*is transmitted via the ingestion of food or water contaminated with the waste of infected individuals [6]. Although, the clinical symptoms of paratyphoid fever are milder than that of typhoid fever but they are indistinguishable [7]. Hence, the term 'enteric fever' is collectively used to describe both fevers [8]. Following an incubation period of 7 days, *Salmonella* infections are characterized by headache, abdominal pain and diarrhea complemented with onset of fever which could be as high as 40°C [9]. If untreated, symptoms may last up to a month with more complicated symptoms for enteric fever may which includes gastrointestinal complications, bradycardia, myalgia, splenomegaly and hepatomegaly [10]. Cases of NTS induced gastroenteritis are self-limiting and may not require chemotherapy except in immunocompromised individuals [1,10].

Typhoid fever is more severe in children and immunocompromised adults particularly those in developing world where hygienic conditions are deplorable [11]. In 2019, approximately 9 million individuals contracted typhoid, resulting in 110,000 annual fatalities [12]. A substantial percentage of reported cases of typhoid fever from United States are from tourists visiting countries where typhoid is endemic [13]. In Nigeria, the prevalence of typhoid varies from 3.9% to 18.6% [14]. A prevalence study by [15] had estimated the prevalence of typhoid fever among food vendors in Southern Taraba State, North East Nigeria to be 3%. This could be a very significant threat to public health. Though antibiotics are a typical treatment for typhoid fever [16]. Neomycin, Ciprofloxacin, Augmentin and Cefotaxime are effective in treating clinical cases of typhoid fever [8]. Nonetheless, escalating antimicrobial resistance poses significant challenges [17]. Chloramphenicol and Fluroquinolones which was a conventional drug of choice against enteric fever and NTS induced gastroenteritis has been resisted by the pathogen [8].

Nonetheless, Since December 2017, two WHO-prequalified typhoid conjugate vaccines have been incorporated into childhood immunization programs in endemic regions [18]. Saly, no vaccine is available yet for the NTS [18]. The typhoid conjugate vaccine is recommended for children aged 6 months and adults up to 45 or 65 years, depending on the specific vaccine [18]. Clinical specimen required for the laboratory diagnosis of typhoid fever is blood and stool samples [19]. Widal tests, blood and stool culture has been documented as efficient laboratory test for detecting *Salmonella* infections in humans[20]. However, the use of PCR targeting specific genes has been recommended as rapid confirmatory tests for typhoid fever[21].

The *invA* gene is a critical virulence factor found in various *Salmonella* serovars[22]. This gene is essential for *Salmonella*'s ability to invade host cells and cause systemic infection [23]. *InvA* is part of the type III secretion system (T3SS), a molecular machinery used by *Salmonella* to inject virulence factors directly into host cells [24]. Mutations or deletions in the *invA* gene significantly reduce *Salmonella*'s invasiveness and pathogenicity, highlighting its importance in the disease process [25]. Hence, this research aims to investigate the prevalence of the invA gene in *Salmonella* strains isolated from human sources in Southern Taraba State, North East Nigeria. Understanding the prevalence of *invA* is crucial for elucidating the molecular epidemiology of *Salmonella*infections in the region. Furthermore, characterizing *invA* in these isolates can provide insights into antimicrobial resistance patterns, essential for guiding antibiotic treatment strategies and the development of effective preventive measures and control strategies for Salmonella *infections* in resource-limited settings

1. METHODS

Study Area: This is a follow-up epidemiological research conducted in Wukari, Donga, Ibi and Takum Local Government Areas located in Southern Taraba State, North-East Nigeria. Southern Taraba has a population of approximately 704,900 citizens. Southern Taraba is bordered by Gassol, Bali, Kurmi, Gashaka, and Ardo- Kola Local Government Areas to the North, Plateau and Nasarawa States to the Northwest, and to the Southeast by Benue State and the Republic of Cameroun. The population in this study are predominantly farmers and traders [8].

Sample collection: As documented in a previous prevalence study by Imarenezor et al (2022), a total of 22 (6 typhoidal, and 16 Non typhoidal) *Salmonella*species was isolated and preserved as stock culture. A sterilized wire loop was used to inoculate from the stock culture into SSA incubated for overnight at 37^{0}C to confirm the isolates. Isolates were further subjected to biochemical analysis. Reference strains including *Salmonella* enterica serovar Typhimurium (strain number 14028), *Salmonella* enterica serovar Enteritidis (strain number 13076), and *Salmonella* enterica serovar Typhi (strain number 19430) were used as quality control measures for isolates in the study.

DNA extraction: The DNA template of the presumed *Salmonella* isolates required for the PCR procedure was extracted by boiling technique. A sterile loop was used to inoculate colony of presumed *Salmonella* isolates from a pure culture medium into a 300µL basic buffer; TE (tris-ethylenediaminetetraacetic acid) contained in a microtube and vortexed. Thereafter, the microtube was boiled for 20 minutes. Afterward the microtube was centrifuged at 12,000 rpm for 10 minutes to obtain the DNA template contained in the supernatant which was immediately transferred to new microtubes.

DNA amplification: PCR was used to amplify the *SalmonellainvA* gene from the DNA template extracted. Reagents amounting to 50µl was used in the amplification process. Reagents included 32.5µl of distilled water, 1 × GoTaq PCR reaction buffer (containing 1.5 mM MgCl2), 2.5 µlDNA template, 0.5µl of 5 units of GoTaq DNA polymerase (gene replication enzyme), 10µl of 1X GoTaq PCR reaction buffer containing 1.5 mM MgCl2, 0.5µl of 0.2 mM PCR nucleotide mix (responsible for unzipping the strand), and 4 µl each of forward and reverse DNA primers (forward (*invA*) '5GTG AAA TTA TCG CCA CGT TCG GGC AA3' and primers reverse (*invA*) '5TCA TCG CACCGTCAA AGG AAC C3'). The mixture of PCR reagent was loaded in a thermo-cycler with an initial denaturation for 5 mins at 94°C. This was followed denaturation for another 1 min at 94°C consisting of 35 cycles. Then, annealing at occurred for 30 s at 58.3°C, and extension for 1 min at 72°C. The final elongation followed at a temperature of 72°C 5 mins. A volume of 3 µl of loading solution (Bromophenol blue and Xylen cyanol) was mixed with 3 µl of amplified products thoroughly. Solution without a DNA template was considered as a negative control.

Electrophoresis of PCR products and band visualization: A mixture of Agarose gel powder and electrophoresis buffer (tris-borate-ethylenediaminetetraacetic acid; TBE) was heated to

dissolve and allowed to cool to about 55^0C. A template for making wells (comb) was positioned in a gel casting chamber. The cooled gel was added with 5µl ethidium bromide and was poured into the tray of the casting chamber and allowed to cool for 20 mins to about 27^0C. The comb was removed, and the gel placed in an electrophoresis chamber and covered with TBE buffer. Using a sterile micropipette, a molecular marker (100 bp) was loaded in the first well followed by the negative control and then the PCR amplicons already mixed with loading dyes which allows amplicons to sink into the gel and provide color for visualization. Electrophoreses was done for 45mins a constant voltage of 100 v. Thereafter gel was transferred to the UV Trans-illuminator where the bands were visualized.

2. RESULTS AND DISCUSSION

Table 1: Primer sequences used in this study

SN	Organism	Virulent gene primers	Amplicon size	Source of primer
1	***Salmonella specie***	***invA*fw GTG AAA TTA TCG CCA CGT TCG GGC AA** ***invA* rev TCA TCG CAC CGT CAA AGG AAC C**	**284bp**	**[26]**

Table 2 presents the prevalence of the invA gene detected among Salmonella isolates obtained from food vendors in various locations. The table indicates that all Salmonella isolates tested positive for the invA gene, with a prevalence of 100% in each location. Specifically, in Wukari, 5 out of 5 isolates were positive for the invA gene; in Donga, 5 out of 5 isolates; in Takum, 5 out of 5 isolates; and in Ibi, 7 out of 7 isolates tested positive for the invA gene. These results indicate a widespread presence of this virulence factor among the isolates collected from food vendors across all studied locations.

Table 2: Prevalence of InvA gene detected among *Salmonella* isolates from food vendors

SN	Location	*Salmonella* isolates				Total *invA gene*	Total *invA* (%)
		TS	*invA gene*	*NTS*	*invA gene*		
1	Wukari	2	2	3	3	5	100%
2	Donga	0	0	5	5	5	100%
3	Takum	1	1	4	4	5	100%
4	Ibi	3	3	4	4	7	100%
	Total	6	6	16	16	22	100%

Figures 1 to 4 display the agarose gel electrophoresis results of PCR amplification products targeting the invA gene in Salmonella isolates from human sources in different locations. In each figure, lanes representing test samples from both Salmonella Typhi (S. typhi) and non-Typhi Salmonella (NTS) are shown, along with a negative control lane.

From the gel electrophoresis results, it can be observed that all test samples, regardless of the Salmonella serotype (Typhi or non-Typhi), displayed PCR amplification products of the expected size (284 bp), indicating the presence of the invA gene. Specifically, in Ibi, all 7 isolates tested positive for the invA gene; in Donga, all 5 isolates; in Takum, all 5 isolates; and in Wukari, all 5 isolates exhibited the presence of the invA gene. Additionally, the absence of amplification in the negative control lane confirms the specificity of the PCR assay.

The results demonstrate a high prevalence of the invA gene among Salmonella isolates obtained from food vendors in the studied locations, suggesting a significant presence of virulent strains in these settings. Furthermore, the detection of the invA gene in all human isolates, irrespective of

the Salmonella serotype, highlights the widespread distribution of this virulence factor among Salmonella strains infecting humans.

Detection of *invA* gene in human isolates

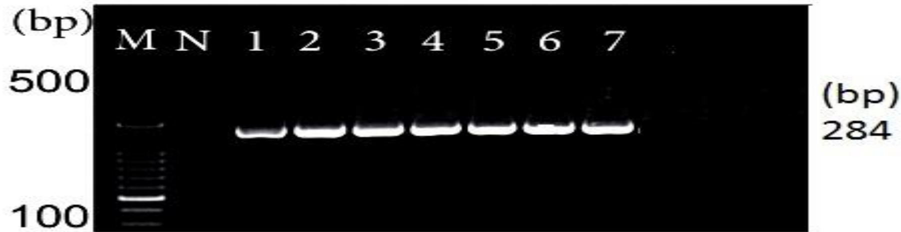

Figure 1: Agarose-gel electrophoresis displaying PCR amplification products of 284 bp *invA* gene in 7 *Salmonella* isolates from human sources in Ibi
Lane M, 100 bp marker; Lane 1–3, test samples (S. typhi); Lane 4 – 7, Test samples (NTS); lane N, Negative control;

Figure 2: Agarose-gel electrophoresis **displaying** PCR amplification products of 284 bp *invA* gene in 5 *Salmonella* isolates from human sources in Donga
Lane M, 100 bp marker; Lane 1–5 Test samples (NTS); lane N, Negative control.

Figure 3: Agarose-gel electrophoresis **displaying** PCR amplification products of 284 bp *invA* gene in 5 *Salmonella* isolates from human sources in Takum
Lane M, 100 bp marker; Lane 1, test samples (*S. typhi*); Lane 2 – 5, Test samples (NTS); lane N, Negative control

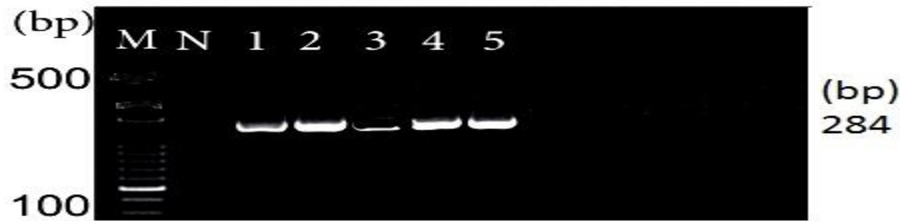

Figure 4: Agarose-gel electrophoresis **displaying** PCR amplification products of 284 bp *invA* gene in 5 *Salmonella* isolates from human sources in Wukari
Lane M, 100 bp marker; Lane 1–2, test samples (S. typhi); Lane 3 – 5, Test samples (NTS); lane N, Negative control.

Polymerase Chain Reaction analysis presented that *invA* gene was detected in all 22 *Salmonella typhi* isolates from food vendors with an approximate amplicon size of 284bp. This is in line with the findings of [21] which detected the *invA* gene in 100% of samples screened and maintained that the *invA* gene is the reference target gene for the detection of *Salmonella*. Supportably, [27] gave that the invasive *invA* gene is found in all *Salmonella* serotypes particularly those that are responsible for systemic infections. Contrastingly, [28] had concluded in earlier research that the amplification of *invA Salmonella* invasive gene is a confirming test for *Salmonella* isolates. Contrastingly the findings of this current study are inconsistent with that of [23] which did not detect *invA gene* in 19 of the 98 *Salmonella* samples subjected to molecular characterization. Although, isolates involved in the study by [23] were not from human source, whereas isolates included in this current study are all from human source (food vendors). Nonetheless, [23]concluded that some strains of *Salmonella* could be or not invasive depending on the possession of any of the invasive genes not limiting to *invA*.

Most invasive gene including the *invA* gene responsible for pathogenicity are predominant in the human cells, and are able to transmit to other potential non-invasive bacteria immediately they gain entrance to the cell except they are immediately acted upon by antibiotic action [29].

Intracellular conjugation of bacteria plays a vital role in the evolution and transfer of the *SalmonellainvA* genes in pathogenic organisms using the horizontally mobile elements (HMEs) as vectors [30]. During the systemic infection of the human cells by *Salmonella typhi*, the microbe is able to acquire virulent and resistance genes inside the mammalian cell from other resident pathogens via conjugative plasmid transfer [31,32]. Hence, the detection of the *invA* gene in all isolates from this current study is significant.

Opposing to the argument by [28], molecular detection of the *invA* gene in *Salmonella* isolates should be complementary rather than being "confirmatory" test for *Salmonella* isolates as suggested by [33]. Therefore, culturally and biochemically confirmed *Salmonella* isolates should not be immediately labelled false positive when the *invA* gene is not detected by PCR. This is because some *Salmonella* isolates may be sensitive to antibiotics action before receiving the *invA* gene from other localized bacteria harboring the gene in the cell [34]. False positives from culturally confirmed isolates reported by previous studies may be due to the sample used for analysis. Stool culture has been observed to be less sensitive than blood culture in detecting *Salmonella typhi* [35]. However, *Salmonella typhi* is more rapidly detected from sample from bone marrow than from blood. This may not be unconnected by the fact that, some researchers may include participants who are already on antibiotic therapy in their study. Hence, blood cultures may not yield bacterial growth following prior antibiotic action [36]. In this current study, positive samples from tube agglutination were subjected to blood culture alongside stool culture for confirmatory results. Therefore, chance for bias was greatly minimized. Bacteriological culture if properly done remains the gold standard for conclusive diagnosis of *Salmonella*[37]. However, bacteriological culture is limited by its long culture period which may extends up to 5 days [38]. Additionally, *invA* negative *Salmonella* isolates from PCR analysis

subsequently tested positive using other techniques [39]. This occurrence was also demonstrated by study by [27] using commercial antisera to successfully serotype*Salmonella* isolates which do not harbor the *invA* invasive gene as confirmed by PCR. Of the 91 serotyped *Salmonella* isolates by [21], only 70 (78%) harbored the *invA* gene. These discrepancies observed between PCR testing for the *invA* gene and alternative techniques, such as serotyping using commercial antisera, underscore the complexities inherent in microbial diagnostics. While PCR is a sensitive and specific method commonly used for detecting specific genes like *invA*, false-negative results may occur due to various factors as earlier highlighted [35]. The findings suggest that alternative techniques, such as serotyping, may complement PCR-based methods by providing additional information about *Salmonella* isolates [40]. Therefore, it is crucial to interpret PCR results cautiously and consider employing multiple complementary techniques to ensure accurate and reliable microbial identification. Further research and validation studies are needed to better understand the reasons behind these discrepancies and to optimize diagnostic approaches for *Salmonella* detection.

4. CONCLUSION

This current study concludes by establishing that the possibility of *Salmonella* isolates from clinical cases of enteric fever harboring the *invA* gene and transferring same to potentially non-invasive via plasmid transfer is probable. The detection of the *invA* invasive gene from *Salmonella* isolates from food vendors has further corroborated the recommendations of other researchers that the amplification of *invA* gene should be complementary to culturally and biochemically confirmed isolates of typhoid fever. This research findings will ultimately be useful in effective health promotion in the food production industry and *Salmonella* surveillance.

ETHICAL APPROVAL

Ethical approval was obtained from the Research Ethics Committee of the Department of Microbiology, Federal University Wukari, Taraba State, Nigeria.

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Authors' contributions

This work was carried out in collaboration among all authors. Author AOA carried out the laboratory experiments, statistical analysis, and wrote the first draft of the manuscriptal. All authors read and approved the final manuscript

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